



Aalborg Universitet

AALBORG UNIVERSITY
DENMARK

Association between neutropenia and IgG antineutrophil antibodies in a case of CD40LG deficiency due to two novel mutations

Assing, Kristian; Nielsen, Kaspar René ; Tenstad, Helene Broch; Jakobsen, Marianne Antonius; Nielsen, Christian; Grosen, Dorte; Hartling, Ulla Birgitte

Published in:
Clinical Case Reports

DOI (link to publication from Publisher):
[10.1002/ccr3.2621](https://doi.org/10.1002/ccr3.2621)

Creative Commons License
CC BY 4.0

Publication date:
2020

Document Version
Publisher's PDF, also known as Version of record

[Link to publication from Aalborg University](#)

Citation for published version (APA):

Assing, K., Nielsen, K. R., Tenstad, H. B., Jakobsen, M. A., Nielsen, C., Grosen, D., & Hartling, U. B. (2020). Association between neutropenia and IgG antineutrophil antibodies in a case of CD40LG deficiency due to two novel mutations. *Clinical Case Reports*, 8(2), 313-316. <https://doi.org/10.1002/ccr3.2621>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal -

Take down policy

If you believe that this document breaches copyright please contact us at vbn@aub.aau.dk providing details, and we will remove access to the work immediately and investigate your claim.



CASE REPORT

Association between neutropenia and IgG antineutrophil antibodies in a case of *CD40LG* deficiency due to two novel mutations

Kristian Assing¹ | Kaspar René Nielsen² | Helene Broch Tenstad¹ | Marianne Antonius Jakobsen¹ | Christian Nielsen¹ | Dorthe Grosen³ | Ulla Birgitte Hartling³

¹Department of Clinical Immunology, Odense University Hospital, Odense, Denmark

²Department of Clinical Immunology, Aalborg University Hospital, Aalborg, Denmark

³Hans Christian Andersen's Children Hospital, Odense University Hospital, Odense, Denmark

Correspondence

Kristian Assing, Department of Clinical Immunology, Odense University Hospital, J.B. Winsloews Vej 4, 5000 Odense C, Denmark.
Email: Kristian.assing@rsyd.dk

Present address

Ulla Birgitte Hartling, Department of Pediatrics, Aarhus University Hospital, Aarhus, Denmark

Abstract

This case suggests a mechanistic rationale for the clinical efficacy of intravenous immunoglobulins (IVIG) in treating CD40 ligand (CD40L) deficiency associated neutropenia as it is the first reported instance of free and cell-bound antineutrophil antibodies in a case of CD40L deficiency, accompanied by a prolonged and clinically severe neutropenia.

KEYWORDS

CD40 ligand deficiency, hyper-IgM syndrome, neutropenia, neutrophil IgG auto-antibodies

1 | INTRODUCTION

The CD40 ligand (CD40L) expressed by *CD40LG* fulfills diverse functions. T cell-expressed CD40L is involved in the stimulation of germinal center B cells, antibody isotype switching and the generation of high-affinity antibodies through the process called somatic hypermutation (SHM). Functional mutations in *CD40LG* result in a hyper-IgM syndrome characterized by normal to elevated IgM levels and low to absent IgG and IgA levels.

2 | CASE PRESENTATION

A 12 months old male patient was referred to our university hospital due to a respiratory infection. Neutropenia, which subsequently lasted for 15 months (with neutrophil concentrations often $<0.05 \times 10^9/L$ (neutropenia grade 4), Figure 1), was diagnosed. The patient was not thrombocytopenic and his erythrocytes were direct antiglobulin test (DAT) negative. Antinuclear antibodies (ANA) were not detected. He received subcutaneous granulocyte-colony stimulating

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2019 The Authors. *Clinical Case Reports* published by John Wiley & Sons Ltd.

factor (G-CSF), according to recommended dosage¹ ($5 \mu\text{g}/\text{kg}/\text{day} = 50 \mu\text{g}$ Neupogen[®]/day), for seven consecutive days, without effect on his neutrophil levels but for a marked increase in his eosinophil counts: $2.2 \times 10^9/\text{L}$ (age-adjusted range: $<0.05 \times 10^9/\text{L}$). A bone marrow biopsy showed lively erythropoiesis, normal thrombocytopoiesis, inhibited neutropoiesis, normal eosinophilopoiesis, and no signs of neoplastic hematologic disease. A possible cellulitis, located to his left instep, responded to amoxicillin and clavulanic acid. After discharge from the hospital, he was given preventive amoxicillin. In the following 10 months, his main complaints were gingivitis, oral ulcers, and mucocutaneous candidiasis, the latter amenable to oral antifungals. Next-generation sequencing, using a custom made in-house panel (Thermo Fischer Scientific) found no mutations in genes associated with severe congenital neutropenia: *ELANE*, *HAX1*, *GFII*, *WAS*, or *G6PC3* but revealed

that the patient was hemizygous for two new genetic variants in *CD40LG*: LRG_141, c.[517G > C; 521delA] p.(Ala173Pro; Gln174fs). The patient's two *CD40LG* variants were confirmed by Sanger sequencing. None of the patient's *CD40LG* variants were described in the dbSNP database at NCBI, nor in HGMD, nor in Exome Aggregation Consortium nor in 1000 Genomes Data. Three (SIFT, PolyPhen-2, and mutationassessor.org) out of six prediction tools rated the missense variant c. 517G > C as probably damaging. The other deletion variant c. 521delA, which caused a frameshift at amino acid position 174 (Gln174fs) and introduced a stop codon at amino acid position 190, was clearly damaging. According to the sequence variant nomenclature: varnomen.hgvs.org, the two *CD40LG* variants are to be described as separate mutations.

The same *CD40LG* variants (in cis) were detected by Sanger sequencing in the patient's mother. The patient had

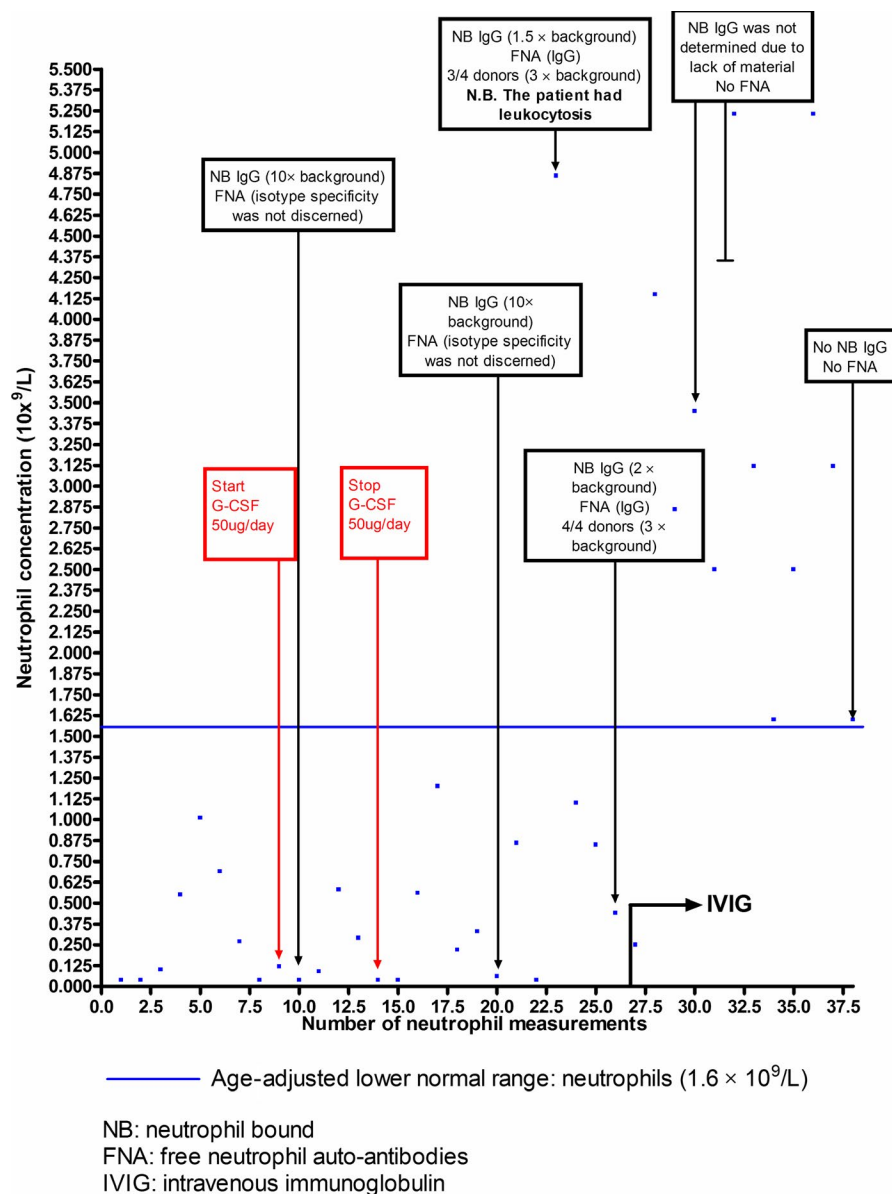


FIGURE 1 The patient's neutrophil concentrations during 28 mo (with 15 mo of neutropenia) divided upon 38 measurements. The blue line depicts the age-adjusted neutrophil concentration (lower normal range). G-CSF ($50 \mu\text{g}/\text{d}$) was administered daily for 7 d. Ten grams of intravenous Privigen[®] were administered every fourth week

undetectable IgG (<0.4 g/L), IgA (<0.05 g/L) but elevated IgM (8.45 g/L) levels consistent with the hyper-IgM syndrome. A lymphocyte marker study, performed at the age of 27 months, showed normal CD4⁺, CD8⁺ T, and CD19⁺ B-cell concentrations but total absence (0%) of isotype switched (CD27⁺IgD⁻) memory B cells (age adjusted 5-95 percentile range: 4.7%-21.2% of CD19⁺ B cells). Patient B cells exhibited reduced SHM (determined by a restriction enzyme-based hot spot mutation assay [REHMA]) of kappa light chain genes: 2% (age-adjusted normal range: >10%, Tissue Typing laboratory, National University Hospital, Copenhagen). *Clostridium tetani* antibody levels (following three tetanus toxoid vaccinations) were nonprotective (<0.01 IU/mL, Statens Serum Institut). Ex vivo stimulation of his CD3⁺ T cells, with phorbol 12-myristate 13-acetate (PMA: 20 ng/mL) and ionomycin (300 ng/mL), failed to upregulate surface CD40L, although his CD3⁺ T cells were activated as evidenced by upregulation of surface CD69. Repeated testing of patient sera by the Luminex-based LABScreen multi-assay (prior to institution of antibody replacement therapy) revealed no antibody specificities toward a wide range of human leukocyte antigen (HLA)-class I, HLA-class II determinants as well as toward human neutrophil antigens (HNAs). Patient sera (prior to and after institution of antibody replacement therapy) and patient neutrophils were tested for granulocyte-reactive antibodies using flow direct-granulocyte immunofluorescence test (D-GIFT), flow indirect-granulocyte immunofluorescence test (I-GIFT), granulocyte agglutinations tests (GAT), and monoclonal-antibody-specific immobilization of granulocyte antigens (MAIGA) as earlier described.² Results were confirmed by another ISBT granulocyte immunology reference laboratory (Karolinska University Hospital). Patient neutrophils were repeatedly (moderately to heavily) covered with IgG (Figure 1) and the corresponding patient sera contained free neutrophil reactive (IgG) antibodies (Figure 1). Possibly reflecting the aftermath of reactive leukocytosis (concomitant leukocyte: $20.5 \times 10^9/L$ (normal range $<14.0 \times 10^9/L$) and lymphocyte concentration: $13.8 \times 10^9/L$ (normal range $<7.9 \times 10^9/L$), but with normal CRP (<1 mg/L), we recorded a single instance where the patient's neutrophil concentration was normalized ($\geq 1.6 \times 10^9/L$), (Figure 1) despite the presence of neutrophil auto-antibodies. The more prolonged normalization of our patient's neutrophil counts coincided with the disappearance of free and cell-bound neutrophil antibodies (measured on three different occasions, Figure 1). While on IVIG (10 g Privigen®/4th week was initiated at the age of 27 months, Figure 1) and prophylactic sulfamethoxazole with trimethoprim, our patient, aged three years and 6 months, underwent successful allogeneic bone marrow transplantation. Written consent by the patient's parents and permission from the chairman of the Regional Committee

on Health Research Ethics for Southern Denmark (case S-20192000-48) was obtained.

3 | DISCUSSION

Neutropenia affects approximately two-thirds of patients with CD40L deficiency, leading to significant comorbidity due to mucocutaneous inflammation.³ The pathogenesis underlying CD40L deficiency associated neutropenia has not yet been clearly delineated. As bone marrow stromal cells express CD40,⁴ stimulation by CD40L can induce them to produce G-CSF.⁵ Hence, CD40L deficiency could compromise the ability of bone marrow stromal cells to secrete G-CSF, thereby causing neutropenia. Supporting that hypothesis, treatment with G-CSF has been reported to increase or normalize the neutrophil counts in CD40L deficiency.⁶ However, seven days of treatment with a standard dosage of G-CSF did not affect our patient's neutropenia but instead markedly increased his peripheral eosinophil concentration, indicating that the G-CSF levels were sufficient to induce myelopoietic lineage differentiation. Still, we cannot exclude that the standard G-CSF dosage was insufficient, since the patient, despite the presence of antineutrophil antibodies, experienced a pronounced, possibly reactive, neutrophil increase, consistent with G-CSF responsiveness. Infection-induced G-CSF secretion results in massive bone marrow neutrophil release⁷ which may override the effects of neutrophil antibodies. Hence, pronounced bone marrow release of neutrophils, secondary to infection, may have effected our patient's transiently normalized neutrophil count in the presence of neutrophil auto-antibodies. Theoretically, our patient could have experienced additional unrecorded instances of normalized neutrophil concentrations, however, his prolonged clinical complaints, involving gingivitis, oral ulcers, and mucocutaneous candidiasis, indicated sustained neutropenia.

The clinical efficacy of IVIG, in treating CD40L deficiency associated neutropenia,⁸ suggests that autoimmune mechanisms might also be involved, although autoimmune neutropenia has never been confirmed in this disorder⁸ and some authors even mention it as unlikely.⁹ We hereby represent the first case of CD40L deficiency, accompanied by undetectable plasma IgG and IgA concentrations, but where free and cell-bound antineutrophil IgG antibodies coincided with a protracted, clinically significant neutropenia. We were not able to determine the subclass specificity of these IgG antibodies, but to varying degrees all IgG subclasses may engage Fc-receptors and thereby contribute to splenic opsonization of antibody-coated neutrophils. The mechanism (s) underlying our patient's sparse production of IgG is uncertain. However, CD40L independent production of IgG toward viral glycoproteins has been documented¹⁰ and neutrophils express several surface glycoproteins, such as CD11b¹¹ and CD47,¹² which could constitute targets for CD40L independent IgG

production. Interestingly, Litinskiy et al¹³ have demonstrated that human dendritic cells can induce CD40L independent IgG class shift through expression of the B-cell stimulator proteins: B-cell activating factor of the tumor-necrosis-factor family (BAFF) and a proliferation-inducing ligand (APRIL). We were not able to confirm the antigen specificity of the IgG antibodies, however, we cannot preclude that the antibodies were “neutrophil specific” as other autoantigen specificities were not detected (no thrombocytopenia, the patient was DAT negative and we observed no ANA reactivity). However, the lack of any known neutrophil antigen specificity of these antibodies concurred with our patient's severely reduced SHM, as CD40L is crucial for SHM. Auto-reactivity is often a feature of compromised SHM, since inherently auto-reactive B-cell clones are prevented from rescuing themselves, through the process of SHM, from auto-reactivity, a process called “clonal redemption”.¹⁴

4 | CONCLUSION

We present a case of CD40L deficiency where the presence of neutrophil reactive IgG coincided with a clinically severe neutropenia. Due to its diverse immunomodulatory capabilities, IVIG therapy is widely used for treating autoimmune disorders. Our case suggests a mechanistic rationale for the clinical efficacy of IVIG in attenuating CD40L deficiency associated neutropenia and substantiates models showing that the requirement for CD40L, in mediating B-cell IgG isotype switching, can be circumvented.

ACKNOWLEDGMENTS

We are grateful for the technical assistance provided by Anne-Louise Fjordside Bilde and for the critical comments provided by Niels Fisker, MD., PhD.

CONFLICT OF INTEREST

The authors have no conflicts of interest relevant to this article to disclose.

AUTHOR CONTRIBUTIONS

Kristian Assing conceptualized the case, collected blood samples and wrote the final paper. Kaspar René Nielsen performed the neutrophil specific auto-antibody assays and critically read the final paper. Helene Broch Tenstad collected blood samples, wrote parts of the initial paper and critically read the final paper. Marianne Antonius Jakobsen performed genetic work-up and critically read the final paper. Christian Nielsen performed flow-cytometric analyses and critically read the final paper. Dorte Grosen organized blood sampling, attended the patient and critically read the final paper. Ulla Birgitte Hartling attended the patient, collected blood samples, wrote parts of the initial

paper and critically read the final paper. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

ORCID

Kristian Assing  <https://orcid.org/0000-0001-8744-1615>

REFERENCES

1. Paul M, Ram R, Kugler E, et al. Subcutaneous versus intravenous granulocyte colony stimulating factor for the treatment of neutropenia in hospitalized hemato-oncological patients: randomized controlled trial. *Am J Hematol*. 2014;89(3):243-248.
2. Bux J, Kober B, Kiefel V, Mueller-Eckhardt C. Analysis of granulocyte-reactive antibodies using an immunoassay based upon monoclonal-antibody-specific immobilization of granulocyte antigens. *Transfus Med*. 1993;3(2):157-162.
3. Seyama K, Nonoyama S, Gangsaas I, et al. Mutations of the CD40 ligand gene and its effect on CD40 ligand expression in patients with X-linked hyper IgM syndrome. *Blood*. 1998;92(7):2421-2434.
4. Franco G, Guarnotta C, Frossi B, et al. Bone marrow stroma CD40 expression correlates with inflammatory mast cell infiltration and disease progression in splenic marginal zone lymphoma. *Blood*. 2014;123(12):1836-1849.
5. Mavroudi I, Papadaki V, Pyrovolaki K, Katonis P, Eliopoulos AG, Papadaki HA. The CD40/CD40 ligand interactions exert pleiotropic effects on bone marrow granulopoiesis. *J Leukoc Biol*. 2011;89(5):771-783.
6. Leven EA, Maffucci P, Ochs HD, et al. Hyper IgM syndrome: a report from the USIDNET registry. *J Clin Immunol*. 2016;36(5):490-501.
7. Christopher MJ, Link DC. Regulation of neutrophil homeostasis. *Curr Opin Hematol*. 2007;14(1):3-8.
8. Levy J, Espanol-Boren T, Thomas C, et al. Clinical spectrum of X-linked hyper-IgM syndrome. *J Pediatr*. 1997;131(1 Pt 1):47-54.
9. Etzioni A, Ochs HD. The hyper IgM syndrome—an evolving story. *Pediatr Res*. 2004;56(4):519-525.
10. Szomolanyi-Tsuda E, Welsh RM. T-cell-independent antiviral antibody responses. *Curr Opin Immunol*. 1998;10(4):431-435.
11. Hill GE, Alonso A, Thiele GM, Robbins RA. Glucocorticoids blunt neutrophil CD11b surface glycoprotein upregulation during cardiopulmonary bypass in humans. *Anesth Analg*. 1994;79(1):23-27.
12. Oldenborg PA. CD47: a cell surface glycoprotein which regulates multiple functions of hematopoietic cells in health and disease. *ISRN Hematol*. 2013;2013:614619.
13. Litinskiy MB, Nardelli B, Hilbert DM, et al. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. *Nat Immunol*. 2002;3(9):822-829.
14. Reed JH, Jackson J, Christ D, Goodnow CC. Clonal redemption of autoantibodies by somatic hypermutation away from self-reactivity during human immunization. *J Exp Med*. 2016;213(7):1255-1265.

How to cite this article: Assing K, Nielsen KR, Tenstad HB, et al. Association between neutropenia and IgG antineutrophil antibodies in a case of *CD40LG* deficiency due to two novel mutations. *Clin Case Rep*. 2020;8:313–316. <https://doi.org/10.1002/ccr3.2621>